SOME PHYSICAL AND NUTRITIONAL FACTORS FOR GROWTH AND SPORULATION OF *CLAVICEPS FUSIFORMIS* LOV.

by R.K. SHARMA, O.P. VERMA and V.N. PATHAK*

SUMMARY. — Effect of some physical and nutritional factors on growth and sporulation of *Claviceps fusiformis* Lov., the incitant of pearl millet ergot, was investigated. A temperature of 25° C was optimum for growth and sporulation of the pathogen. Maximum growth and sporulation were obtained respectively, at pH 7,5 and 6.5. Sporulation was checked at pH 9.0, not the growth. Sugar level in the basal medium below 100 g/l caused significant reduction in growth and sporulation. Asparagine and magnesium sulphate were best sources of nitrogen and sulphur, respectively. Sulphates of barium, bismuth, sodium, copper and ferrus caused complete inhibition of the fungal growth.

RÉSUMÉ. — Les effets de quelques facteurs physiques et nutritionnels sur la croissance et la sporulation de *Claviceps fusiformis* Lov., l'ergot du millet perlé, sont étudiés. Une température de 25° C est optimale pour la croissance et la sporulation du pathogène. Une croissance et une sporulation maximales ont été obtenues respectivement à pH 7,5 et 6,5. La sporulation est stoppée à pH 9,0, mais pas la croissance. Une concentration de sucre inférieure à 100 g/l dans le milieu produit une réduction significative de la croissance et de la sporulation. L'asparagine et le sulfate de magnésium semblent les meilleures sources d'azote et de soufre. Les sulfates de barium, de bismuth, de sodium, de cuivre et de fer produisent l'inhibition complète de la croissance fongique.

MOTS CLÉS : *Claviceps fusiformis*, croissance, sporulation.

A thorough knowledge of physiology of a pathogen pave the way for better understanding of host-parasite relationship which ultimately helps in devising effective control measures. Such studies become more important with a pathogen like *Claviceps fusiformis* Lov. where the pathogen causes severe losses to the crop (*Pennisetum americanum* (L.) Leeke) (SACCAS, 1954; RAMASWAMY, 1968; KING, 1975; NENE & SINGH, 1976) as well as poses health hazards (PATEL & al., 1958; SHONE & al., 1959; KRISHNAMACHARI & BHAT,

* Department of Plant pathology, Sukhadia University, SKN College of Agriculture, Jobner 303329, India.

1976). In this communication, effect of certain physical and nutritional factors on growth and sporulation of the pearl millet ergot pathogen are reported.

MATERIALS AND METHODS

In all the experiments, Kirchhoff's medium (Sucrose, 100 g; potassium dihydrogen phosphate, 1.0 g; asparagine, 1.0 g; magnesium sulphate, 0.25 g; distilled water, 1.0 l) was employed because of its suitability to *Claviceps* spp. (KIRCHHOFF, 1929; SHINDE & BHIDE, 1958; SHARMA, 1980). Except experiment with H-ion concentration, pH of the medium was buffered to 7.0 before autoclaving. Twenty five ml of sterilized medium contained in each of 100 ml conical flask was inoculated by 2 mm diameter disc cut out from the periphery of 20-day-old single-spore-culture of the pathogen. The inoculated flasks were incubated at 25 ± 1°C (except those of temperature experiment), for 20 days. After incubation, the fungal growth was filtered through Whatman filter paper No. 44 of known weight, dried to constant weight and weighed to find out net weight of the fungal growth.

Five flasks were inoculated in each treatment. Of these, three were used as three replications for measuring the growth. Contents of the remaining two flasks were mixed thoroughly and used for sporulation count using haemocytometer. Mean of 10 observations was recorded as one count and such three counts were made to make 3 replications of a treatment. Other specific details of individual experiments are as under.

– Effect of sugar concentration : Sucrose concentrations in the basal medium were adjusted to 50, 75, 100, 125 and 150 g/l.

– effect of temperature : Inoculated flasks were incubated at seven temperatures viz., 7, 15, 20, 25, 30, 35 and 40°C for 20 days.

– Effect of H-ion concentration : The basal medium was buffered before autoclaving to different pH levels ranging from 5.0 to 9.0 with a difference of 0.5.

– Effect of nitrogen compounds : From the basal medium, asparagine was replaced by eight different nitrogen compounds viz. : ammonium oxalate, ammonium sulphate, ammonium carbonate, ammonium nitrate, ammonium ferrus sulphate, potassium nitrate, sodium nitrate and urea, so that nitrogen supplied by these compounds was equivalent to that supplied by asparagine in the basal medium.

– Effect of sulphur compounds : From the basal medium, magnesium sulphate was replaced by nine different sulphur compounds viz., ammonium sulphate, barium sulphate, bismuth sulphate, calcium sulphate, copper sulphate, ferrus sulphate, potassium sulphate and zinc sulphate. Each compound to supply the amount of sulphur equivalent to that supplied by magnesium sulphate in the basal medium.

OBSERVATIONS

The five treatments correspond to different experiments, so that in Tables 1 to 5, results corresponding to some experimental conditions (basal medium, temperature) are not absolutely similar.

– Effect of sugar concentration : Increase in sugar concentration increased growth of the pathogen (Table 1). Although maximum growth was recorded in the treatment having 150 g sugar/l, it was nearly to that in treatments having 100 and 125 g/l. Lowest concentration of sugar produced lowest growth. Sugar concentration of 100 g/l and more gave significantly more sporulation as compared to lower concentration.

Sugar level in medium (g/l)	Average dry weight of the mycelium (mg)	Number of spores (Millions/ml)
50	676	12.60
75	755	16.00
100	890	22.00
125	904	19.00
150	905	21.30
L.S.D. (0.05)	16.26	6.65

Table 1. – Growth and sporulation of *C. fusiformis* on Kirchhoff's medium with different sugar levels.

Tableau 1. – Croissance et sporulation de *C. fusiformis* sous différentes concentrations de sucre (milieu de Kirchhoff).

– Effect of temperature : Temperature of 25°C was proved to be optimum for growth and sporulation of the pathogen (Table 2). Both growth and sporulation were significantly more at this temperature than at any other temperature. Deviation from 25°C resulted in decline of both growth and sporulation.

Temperature (°C)	Average dry weight of the mycelium (mg)	Number of spores (Millions/ml)
7	78	4.60
15	217	8.00
20	607	16.30
25	716	18.60
30	661	15.00
35	457	11.30
40	211	7.30
L.S.D. (0.05)	37.46	1.47

Table 2. – Growth and sporulation of *C. fusiformis* on Kirchhoff's medium incubated at different temperatures.

Tableau 2. – Croissance et sporulation de *C. fusiformis* soumis à différentes températures (milieu de Kirchhoff).

— Effect of H-ion concentration : The fungus could grow on a wide pH range of 5.0 to 9.0 but did not sporulate at pH above 8.5 (Table 3). The optimum pH for growth and sporulation were 7.5 and 6.5 respectively.

pH	Average dry weight of the mycelium (mg)	Number of spores (Millions/ml)
5.0	110	8.30
5.5	203	11.30
6.0	407	12.30
6.5	687	20.00
7.0	740	18.60
7.5	773	17.60
8.0	464	5.60
8.5	275	1.30
9.0	227	0.00
L.S.D. (0.05)	13.74	1.71

Table 3. — Growth and sporulation of *C. fusiformis* on Kirchhoff's medium with different pH levels.

Tableau 3. — Croissance et sporulation de *C. fusiformis* sous différents pH (milieu de Kirchhoff).

— Effect of nitrogen compounds : Of the 9 organic and inorganic sources of nitrogen, asparagine supported maximum growth and sporulation of the fungus (Table 4). It was closely followed by ammonium oxalate. Ammonium ferrus sulphate, ammonium carbonate and urea did not allow the fungus to grow. Nitrogen supplied through nitrates (ammonium nitrate, sodium nitrate and potassium nitrate) supported poor growth and sporulation.

Nitrogen compound	Average dry weight of the mycelium (mg)	Number of spores (Millions/ml)
Asparagine	890	22.30
Ammonium oxalate	730	16.30
Ammonium sulfate	355	6.60
Ammonium nitrate	280	4.30
Sodium nitrate	260	3.30
Potassium nitrate	240	2.60
Ammonium ferrus sulphate	nil	nil
Ammonium carbonate	nil	nil
Urea	nil	nil
L.S.D. (0.05)	14.55	1.95

Table 4. — Growth and sporulation of *C. fusiformis* on Kirchhoff's medium supplemented with different nitrogen compounds.

Tableau 4. — Croissance et sporulation de *C. fusiformis* sous différentes sources d'azote (milieu de Kirchhoff).

— Effect of sulphur compounds : Maximum and significantly more growth and sporulation were recorded when sulphur was supplied through magnesium sulphate (Table 5). It was followed by calcium sulphate. Although there was significant difference in amount of the fungal growth produced by ammonium sulphate, potassium sulphate and zinc sulphate the extent of sporulation in these three treatments was analogous. Barium sulphate, ferrus sulphate, sodium sulphate, copper sulphate and bismuth sulphate caused complete inhibition of the fungal growth.

Sulphur compound	Average dry weight of the mycelium (mg)	Number of spores (Millions/ml)
Magnesium sulphate	880	22
Calcium sulphate	570	17
Ammonium sulphate	430	12
Potassium sulphate	360	14
Zinc sulphate	317	12
Barium sulphate	nil	nil
Ferrus sulphate	nil	nil
Copper sulphate	nil	nil
Bismuth sulphate	nil	nil
L.S.D. (0.05)	10.22	2.05

Table 5. — Growth and sporulation of *C. fusiformis* on Kirchhoff's medium supplemented with different sulphur compounds.

Tableau 5. — Croissance et sporulation de *C. fusiformis* sous différentes sources de soufre (milieu de Kirchhoff).

DISCUSSION

Higher sugar levels in the medium increased the growth and sporulation of the fungus. NAGARAJAN & SARASWATHI (1974) also harvested luxuriant growth and heavy sporulation of sorghum ergot pathogen from modified Kirchhoff's medium having more sugar content. Temperature greatly influenced the growth and sporulation of *C. fusiformis*. The results of the present investigations are parallel to those reported by KREBS (1936). In his investigations, 3 and 24°C were respectively minimum and optimum temperatures for *C. microcephala*. Observations of SIDDIQUI & KHAN (1973) and GAUR & al. (1975) that temperature below 30°C is optimum for development of pearl millet ergot may partly be due to favourable growth and sporulation of the pathogen below 30°C.

Results obtained for growth of the pathogen on different pH are close to those reported by JAT (1976) for *C. microcephala*, but for sporulation, isolate showed lower optimum pH. Another isolate of *C. microcephala* used by KREBS (1936) produced optimum growth at pH 5.40 to 6.33. Such differences in isolates is also common in other fungal species.

Results of nitrogen sources are similar to those obtained by JOHANNA-WESTER (1947), CELAYETA (1961) and THIND & MADAN (1973) concluding asparagine as a better source of nitrogen than nitrates or ammonia for *Claviceps* spp. In the present investigations, response to different nitrogen sources was similar for growth and sporulation, whereas according to JAT (1976) some sources supporting good growth did not support good sporulation of *C. microcephala*. In his investigations, urea supported mycelial growth but not sporulation. Under present investigations, urea did not support even growth of the fungus. Such variation in result may partly be due to differences in isolates of the fungus.

Interesting data were obtained when sulphur was supplied to *C. fusiformis* through different sources. Only five sources supported growth and sporulation whereas barium sulphate, ferrus sulphate, copper sulphate and bismuth sulphate checked the growth completely. Since amount and form (SO_4) of the sulphur supplied were the same by different sources, the differences in growth and sporulation can be attributed to Bi, Cu, Fe, Ba, Zn, K, Ca, Mg and NH_4. It is likely that the amount of Ba, Fe, Cu and Bi was sufficiently more to be toxic to the fungus.

Possibilities of sulphur and nitrogen sources which caused complete inhibition of the fungal growth may be explored for the disease management. This may either be achieved by application of these compounds on host plant or by selection of host genotypes having higher content of one or more of these compounds.

The authors are thankful to the Associate Dean of this campus for providing necessary facilities.

REFERENCES

CELAYETA F.D., 1961 — Comparative study of the influence of different nitrogen and carbon sources on the growth of *Sphacelia segetum* Lev. *Ann. Acad. Farm. Moderno* 27 : 245-261.

GAUR S.C., GOYAL J.P. and PATHAK V.N., 1975 — Forecasting ergot of bajra. Proc. Symp. Pl. Dis. Prob., Udaipur, Sept. 18-20, 1975 (Abstr.) : 52.

JAT R.G., 1976 — Studies on Bajra ergot. Thesis, University of Udaipur, College of Agriculture, Jobner.

JOHANNA-WESTER D., 1947 — On the contribution of fungi in pure cultures. Antonie Von Leeuwenhoek. *J. Microbiol. Serol.* 12 : 223-231.

KING S.B., 1975 — Downy mildew and ergot of pearl millet. *In* : WILLIAMS R.J., Proc. consultant's gp meetings on downy mildew and ergot of pearl miller, ICRISAT : 97-101.

KIRCHHOFF H., 1929 — Contributions to the biology and physiology of the ergot fungus. *Centralbl. Bakteriol., 2.Abth.* : 310-369.

KREBS J., 1936 — Untersuchungen uber den Pilz des Mutterkorns *Claviceps purpurea* Tul. *Ber. Schweiz. Bot. Ges.* 45 : 71-165.

KRISHNAMACHARI K.A.V.R. and BHAT R.V., 1976 — Poisoning by ergoty bajra (pearl millet) in man. *Indian J. Med. Res.* 64 : 1624-1628.

NAGARAJAN P. and SARASWATHI V., 1974 — Production of «Honey-Dew» like secretions in cultures of *Sphacelia sorghi*. *Indian Phytopathol.* 28 : 110.

NENE Y.L. and SINGH S.D., 1976 — Downy mildew and ergot of pearl millet. *PANS* 22 : 366-385.

PATEL T.B., BOMAN T.J. and DALLAL U.C., 1958 — An epidemic of ergot poisoning through ingestion of infected bajra in southern parts of Bombay State. *Indian J. Med. Sci.* 12 : 257-261.

RAMASWAMY C., 1968 — Meteorological factors associated with the ergot epidemic of bajra (*Pennisetum*) in India during Kharif season, 1967 - A preliminary study. *Curr. Sci.* 37 : 331-335.

SACCAS A.M., 1954 — The parasitic fungi of sorghums (*Sorghum vulgare*) and millets (*Pennisetum typhoideum*) in French Equatorial Africa. *Agron. Trop. (Nogent)* 9 : 647-686.

SHARMA R.K., 1980 — Studies on ergot of pearl millet. Thesis, University of Udaipur, College of Agriculture, Jobner, 47 p.

SHINDE P.A. and BHIDE V.P., 1958 — Ergot of Bajri (*Pennisetum typhoides*) in Bombay State. *Curr. Sci.* 27 : 499-500.

SHONE D.K., PHILLIP J.R. and CHRISTIE G.J., 1959 — Agalactia of sows caused by feeding the ergot of the bulrush millet *Pennisetum typhoides*. *Veterin. Rec.* 71 : 129-132.

SIDDIQUI M.R. and KHAN I.D., 1973 — Dynamics of inoculum and environment in relation to ergot indicence on *Pennisetum typhoides* (Burm.) Stapf and Hubbard. *Trans. Mycol. Soc. Japan* 14 : 280-288.

THIND K.S. and MADAN M., 1973 — Effect of various carbon and nitrogen sources on the growth and sporulation of *Claviceps microcephala*. *Proc. Indian Acad. Sci. B*, 78 : 241-256.